Gnotobiotics and immunopathology: The use of the gnotobiotic environment to study acquired and inherited immunodeficiency diseases

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Abstract

Gnotobiotic animals are highly valued for the study of infectious diseases wherein the clinical signs and lesions of disease can be directly related to host–pathogen interactions and not to the additive effects of environmental influences and other confounding factors. Gnotobiotic dogs have been used to study the pathogenesis of acquired immunodeficiencies associated with canine distemper virus (CDV). In recent years, the laboratory at OSU, in conjunction with University of Pennsylvania personnel have begun a series of long-term studies of dogs affected with the canine X chromosome-linked severe combined immunodeficiency (XSCID) syndrome. This fatal inherited defect is caused by mutation in the common gamma chain (IL2RG) gene and renders affected animals profoundly immunodeficient. XSCIDs dogs, raised within a gnotobiotic environment for up to 3 years remain clinically healthy and are, in every respect normal except for the persistent T-cell defect and the failure to develop lymph nodes.

Bone marrow transplantation (unfractionated or enriched for CD34+ stem cells) is the treatment of choice for both the XSCID dogs and male human infants affected with this syndrome. In preliminary studies, we have shown that human CD34+ stem cells colonized XSCID-affected gnotobiotic dogs, migrated to the thymus and demonstrated post-thymic activation (CD45RA+ phenotype) in peripheral blood. While many issues are unresolved, these data suggest that, through the use of the gnotobiotic environment, xenotransplantation (human-to-dog) may yield a stable and immunologically functional human–dog chimera.

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1. Introduction

Animal research using gnotobiotic technologies was initiated at The Ohio State University by Richard A. Griesemer in 1959. The Gnotobiotic Life Laboratory is contained within the Department of Veterinary
Biosciences, College of Veterinary Medicine and has been in continuous operation since that time. Animal care is supervised by University Laboratory Animal Resources (ULAR) and the facility is regularly inspected (announced and unannounced) by the USDA. Under Dr. Griesemer’s direction, methods for construction, preparation and sterilization of equipment were designed, diets for companion animal species were developed and procedures for manipulation of animals in the isolation units were perfected. At its peak of operation in the early and middle 1960s, the laboratory employed up to 10 technical staff and numerous graduate students. During the early phases of operation (1959–1963), the basic methodology for raising gnotobiotic animals was developed. Then, the chief difficulty encountered was procurement of sterile yet nutritious diets for the experimental animals (Griesemer and Gibson, 1963). Throughout the decade of the 1960s, studies of various infectious diseases utilizing gnotobiotic animals were emphasized and major equipment purchases were completed.

Canine diseases investigated included congenital ascariasis (Griesemer et al., 1963), viral infectious diseases such as canine distemper virus (Gibson et al., 1965) and canine reovirus infection (Holzinger and Griesemer, 1966), and fungal diseases such as histoplasmosis (Del Favero and Farrell, 1966). Feline viral infectious diseases studied were panleukopenia (Rohovsky and Griesemer, 1967), rhinotracheitis (Hoover et al., 1970), infectious peritonitis (Wolfe and Griesemer, 1966) and feline leukemia (Hoover et al., 1973).

Porcine diseases studied include porcine poliomyelitis (Holman et al., 1966) and adenovirus encephalitis (Kasza, 1966). In all of these studies, the basic approach was to compare the pathogenesis of infection in gnotobiots versus conventional animals. These studies confirmed that the viral infectious diseases were largely asymptomatic infections in animals devoid of environmental commensal microbes and provided the research community with tissues, monoclonal polyclonal reagents and a composite picture of these disease processes in its simplest form.

In 1971, a major change in direction within the laboratory was made. Formerly, gnotobiology technology was emphasized. Now studies on the pathogenesis of canine distemper virus-associated demyelinating encephalitis in gnotobiotic dogs were performed (Krakowka et al., 1985). Utilization of germfree dogs was essential but of secondary importance to project goals directed toward resolution of the mechanism(s) of demyelination as it may be related to multiple sclerosis and other persistent viral infectious diseases of humans and to the delineation of the immunosuppressive effects of this virus infection upon canine immune functions (Krakowka et al., 1980a,b; Krakowka, 1982). In the 1980s emphasis was changed from gnotobiotic dogs to gnotobiotic swine, first to evaluate oral antibody therapies for rotavirus diarrhea (Cordle et al., 1991) and then to the development and exploitation of gnotobiotic swine infected with Helicobacter pylori as a model for human disease (Krakowka et al., 1987, 1998; Krakowka and Eaton, 1996). In the 1990s and through the present, a series of investigations into the pathogenesis of porcine circovirus type 2, a newly emergent swine pathogen, were conducted (Ellis et al., 1999). The decision to utilize germfree resources as a research tool to investigate specific disease processes in vivo has been vindicated by the successful competitive grant and contract support for the facility through to the present day.

The techniques of gnotobiology seem cumbersome, expensive and unnecessary to the casual observer. However, this perception is balanced by the frustrations encountered in attempting to delineate pathogenic mechanisms of infectious diseases in animals compromised by previous environmental exposure to commensal microbes or rendered immune or actively diseased through exposure to specific or cross-reactive infectious agents. The following advantages (Griesemer, 1965) for using germfree versus conventional animals have been recognized:

1. gnotobiotic animals are more uniformly susceptible to infectious agents and give more uniform responses to infection;
2. gnotobiotes are not infected with intercurrent diseases;
3. new or subtle lesions can be detected in gnotobiots and the pathogenesis of these lesions can be delineated;
4. direct comparisons of virulence of strains of agents can be meaningfully compared in gnotobiotes;
5. the influence of microbial flora upon intestinal infections or chemical carcinogens can be
evaluated, the role(s) of systematic acquisition of normal flora upon innate immune mechanisms can be determined and;

(6) exposure of technical personnel, control (uninfected animals) or the environment is reduced or effectively eliminated when the studies are conducted within the isolation units.

Offsetting these advantages are several disadvantages. Included in this category are:

(1) the one-time expenses incurred in purchasing equipment;
(2) the recurrent expenses for equipment maintenance and the need for specialized equipment such as oversized autoclaves;
(3) the numbers of animals which can be raised for use in the facility are space- and equipment-limited;
(4) for certain species (dogs and cats), hand-rearing to weaning age is labor-intensive and;
(5) the general disadvantage that gnotobiotes may have aberrant innate immune functions and host resistance mechanisms to normal flora due to the lack of both exposure and sub-clinical stimulation by normal microflora.

Finally, specially trained personnel are needed to construct and maintain the isolation units and to conduct studies with germfree animals within the isolation units.

In this short review, the value of gnotobiotic technology is illustrated by the use of this facility to perform long-term experiments with Beagle/Basset dogs affected with X-linked severe combined deficiency disease (XSCIDs), a well characterized canine immunodeficiency disease syndrome (Felsburg et al., 1999, 2003; Jezyk et al., 1989). The defect is due to a four base deletion, which results in a frame shift mutation and premature termination codon in exon 1 of common gamma chain gene (Henthorn et al., 1994). The resultant defective protein (21 amino acid residues versus the normal 373 amino acid residues) is not functional and all cytokine receptors which use the common gamma chain (IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21) are affected by this mutation. Affected dogs are born with absent or low numbers of nonfunctional T-cells but with normal levels of B-cells. Under ordinary circumstances, affected dogs experience repeated infections as maternal immunity wanes and will eventually die of sepsis unless transplanted with histo-compatible canine bone marrow cells or enriched bone marrow origin canine CD34+ stem cells (Felsburg et al., 1995). The XSCIDs dog exactly replicates the most common form of human inherited immunodeficiency disease in male infants (Rosen et al., 1995). The treatment of choice for both XSCID dogs and boys is allogenic bone marrow transplantation (Roberts et al., 1989; Rosen et al., 1995; Felsburg et al., 1995). This manipulation was successfully performed in young adult XSCID gnotobiotic dogs at OSU and in weanling conventional XSCID dogs at the University of Pennsylvania.

1.1. Immunologic profile of the XSCIDs defect

The XSCIDs dog is profoundly and persistently lymphopenic (Felsburg et al., 1999, 2003). The lymphopenia is attributable to the absence of CD3+ T-cells: B-cell numbers are normal or slightly elevated. The defective gamma chain also renders the IL-7 receptor for stem cell growth factor on stem cells nonfunctional, which further contributes to the paucity of T-cells. Thymuses from conventional XSCIDs dogs are hypoplastic and contain less than 3.0% of the number of thymocytes present in age-matched controls (Fig. 1). Thymuses from XSCIDs gnotobiotes are also hypoplastic but the number of thymocytes present in age matched gnotobiotic XSCIDs dogs is 30-fold less than conventional XSCIDs dogs and is only 0.1% of normal age-matched dogs (Felsburg et al., 2003). Conventional XSCID dogs maintain low levels of activated T (CD45RA+) cells, presumably through a thymus-independent T-cell proliferative pathway. In contrast, gnotobiotic XSCIDs dogs do not exhibit any increase in activated (CD45RA+) T-cells over time. This difference is tentatively attributed to the lack of environmental stimulation in the latter. Collectively, these data are compatible with an as yet uncharacterized gamma chain independent pathway for T-cell differentiation, which may be modulated by environmental antigenic signals. In the thymus, the lack of a functional T-cell receptor for IL-2 on XSCIDs thymocytes limits proliferation of pre-thymic and thymic T-cells and impedes their differentiation within
the thymus. However, the transition from the double positive (CD4+CD8+) phenotype to single positive (CD4+CD8− or CD4−CD8+) phenotypes in the thymus, a proliferation-independent differentiation step, appears to be unaffected by the gamma chain defect.

Because Ig class switching in B-cells is largely dependent upon T-cell help or cytokines, both gnotobiotic and conventional XSCIDs dogs have low or absent levels of circulating IgG. Trace amounts of IgG are present in conventional XSCIDs dogs, perhaps because of environmental antigen-mediated direct interactions between dendritic cells and B-cells (Moore et al., 1999). However, immunization studies with a T-cell-dependent B-cell antigen have demonstrated only antibodies of the IgM class in post-immunization sera (Felsburg et al., 1999). Roughly 15.0% of XSCIDS-affected boys lack detectible natural killer (NK) cell activity (Buckley et al., 1997). Limited studies have shown that NK activity is missing in conventional XSCIDs dogs but not in gnotobiotic XSCIDs dogs. In fact, NK responses in gnotobiotic XSCIDs PBMC is greater than that of age-matched normal gnotobiotics (Table 1).

Finally, both conventional and gnotobiotic XSCIDs dogs lack lymph nodes; lymphoid organs in immunologically normal gnotobiotic dogs are hypoplastic because of lack of environmental stimulation (Griesemer and Gibson, 1963; Krakowka et al., 1980a,b) yet are still present, even in dogs affected with canine distemper virus-associated systemic lymphoid depletion and immunosuppression (Gibson et al., 1965; Krakowka et al., 1980a,b, 1985). In mice, proper embryogenic development of lymph nodes requires both lymphotoxin (LT) and its membrane form (tumor necrosis factor alpha). During embryogenesis, B-cell origin LT supports the formation of B- and T-cell zones through the induction of various adhesion molecules (E-selectin, vascular cell adhesion molecule, intracellular adhesion molecule) and peripheral lymph node addressin on endothelia (Chaplin and Fu, 1998; Ruddle, 1999). These B-cell cytokines provide support to the follicular dendritic cell network and thus primary B-cell follicle structure as well. If the
same general mechanism of lymph node genesis is operable in dogs, then a lack of lymph nodes in XSCIDs dogs, even after correction by bone marrow transplants, suggests that T-cell tropic effects upon B-cell development during embryogenesis is ultimately the critical missing component for normal lymph node development in dogs.

1.2. Therapy for the XSCIDs defect

As indicated, allogenic canine bone marrow transplantation is the treatment of choice for the functional correction of the canine XSCIDs defect (Felsburg et al., 1995, 2003). Supportive measures, in the absence of transplantation, provide temporary benefit but are ultimately unsuccessful. Unfractionated marrow and enriched CD34+ stem cell fractions from bone marrow reconstitute T-cell numbers and mitogenic proliferative responses by 60 days after transplantation (Felsburg et al., 2003). This delay in T-cell regeneration is of considerable importance since the animals are profoundly immunodeficient during this interval. For this reason, the sooner that the marrow transplants can be performed (2–3 weeks of age while maternal immunity is still present) the more likely it is that a successful outcome will be achieved. Since pre-transplant conditioning (irradiation or chemical ablation) is not used, the reconstituted dogs are fertile. One immediate benefit of successful transplantation is the creation of carrier male dogs which reach breeding age. When bred to a phenotypically normal carrier female, an expected one-half of the female puppies will be homozygous for the gamma chain defect. Marrow transplantation of these XSCIDs-affected females and breeding these dogs to marrow-reconstituted XSCIDs-positive males will produce progeny with a 100% incidence of the XSCIDs defect. This breeding strategy has greatly increased the number of animals for study. Finally, it was recently shown that the post-transplant immunosuppressive interval could be reduced by one-third with recombinant IL-7 (stem cell growth factor) therapy (Felsburg et al., 2003). This approach suggests that supplementation of recipients with IL-7 will be of benefit to human patients who need marrow reconstitution after a diagnosis of XSCIDs defect or after marrow ablation therapies.

1.3. Are canine marrow-transplanted XSCIDs dogs immunologically normal?

Only a partial and qualified “yes” to this crucial question is available at the moment. Certainly, when compared to their chances without reconstitution, marrow transplantation is a life-saving event. This aside however, certain problems accompany long-term transplanted dogs. Firstly, lymph nodes are not formed, even though the PBMC profiles in transplants eventually resemble the normal age-matched canine controls (Felsburg et al., Unpublished data, 2005). Persistent bacterial dermatitis is often seen. Again, both conditions again suggest that there are persistent defects in immune and/or innate responses on body surfaces. Preliminary studies suggest that the T-cell repertoire of transplanted dogs declines over time, in a fashion compatible with premature immunologic senescence (Felsburg et al., 2003). Finally, since the gamma chain deficiency is global.
and not restricted to the IL-2 receptor on T-cells, subtle
defects in other gamma chain-dependent cytokine
signaling pathways are expected. However, the
apparent redundancy of the signaling network
suggests that these defects, if present, may be
circumvented or over-ridden by alternate pathways.

1.4. The XSCIDs defect: reconstitution with
human CD34+ stem cells

There is no reason why immunologic reconstitution
of XSCIDs dogs cannot be accomplished with
xenografts (out-of species or human) sources of bone
marrow and/or CD34+ stem cells. Theoretically,
human stem cell (HSC)-origin CD34+ cells will
develop in the thymus and will recognize antigens
only in the context of MHC determinants unique to the
thymic microenvironment of the canine XSCIDs
recipient rather than the genotype of the human donor
(Roberts et al., 1989; Geha and Rosen, 1989).
Therefore, human T-cell precursors, under the
influence of the XSCID canine thymic microenviron-
ment, will not recognize dog as “foreign” and graft
rejection of the canine host (graft versus host reaction)
will not occur. Moreover, the XSCID dog cannot reject
transplanted human HSC since recipient XSCIDs dogs
are T-cell deficient and thus cannot mount an immune
response to antigens (host versus graft reaction)
expressed on HSC and their differentiated progeny.
Thus, there is every reason to believe that human
marrow engraftment and successful immunologic
reconstitution can be achieved in XSCIDs dogs.
Preliminary data support this belief.

In 2003, two 3-week-old gnotobiotic (Krakowka et al., 1978, 1980a,b) XSCIDs dogs were transplanted
with human enriched CD34+ cells by injection of
$1.5 \times 10^6$ cells into the femoral bone marrow space.
Successful thymic engraftment was monitored by
serial evaluation of collected PBMC for expression of
human CD4+ and CD45RA+ (recent thymic emi-
grants) cell populations by flow cytometry using
monoclonal reagents specific for human CD antigens.
Circulating cells of human T-cell phenotypes were
detected in one dog 6 weeks after engraftment and in
the other 8 weeks after engraftment. While the
absolute numbers of circulating lymphocytes
remained low (roughly 1000 lymphocytes versus 4–
5000 lymphocytes in normal gnotobiotic dogs) and the
dogs remained lymphopenic throughout the observa-
tion period (18 weeks post-transplantation), there was
no doubt that these cells were of human origin.
Curiously in both dogs, cyclic fluctuations in the
absolute numbers of circulating human cells were also
seen. These data suggest that the reconstituted thymus
periodically released new human T-cells (CD45RA+)
into the circulation but, in the absence of post-thymic
antigenic stimulation in the gnotobiotic environment,
these gradually disappeared from the circulation,
presumably by apoptosis. This decline was then
followed by a second (and ultimately 4–5) bursts of
newly generated CD45RA+ T-cells.

Rather than immunize or conventionalize these
chimeric human XSCIDs dogs, it was decided to
euthanatize them 20 weeks after transplantation and
examine lymphoid tissues for histologic and immu-
nohistologic evidence of colonization by the engrafted
human cells. As with canine marrow transplants,
lymph nodes were not found. The XSCIDs thymus
(Fig. 1, right panel) is an epithelial structure encased in
the anterior mediastinum and characterized by the
absence of cortical or medullary thymocytes, except
for occasional small “islands” of thymocytes in the
cortex. There is no histologic evidence of thymic
epithelial keratinization in the XSCIDs thymus. In
contrast to the XSCIDs control thymus, the thymus of
a dog reconstituted with human CD34+ cells (Fig. 2)
contained variable but plentiful numbers of thymo-
cytes, presumably of human T-cell origin. Afferent
lymphatics in corticomedullary areas are distended
with mature lymphocytes suggesting that there is
active exiting of T-cells from the thymus to the
periphery.

There are other points to be made regarding the
histologic appearance of the reconstituted thymus.
Firstly, unlike normal thymus, thymic cortices of the
reconstituted dogs were discontinuously colonized by
thymocytes (Fig. 2, right panel). In some areas, the
thymic cortex was devoid of thymocytes. This pattern
(segmental cellularity) is reminiscent of embolic
vascular events and strongly suggests that each focus
of thymocyte development in the thymus was the
result of colonization by one (or only a few) human
CD34+ stem cells released from bone marrow. If this
view is correct, it is possible that this phenomenon is
responsible for the cyclic fluctuations in peripheral
blood T-cell numbers in these dogs. As thymocytes
derived from these single stem cells develop and proliferate, the areas of colonization become populated with thymocytes. As they exit, the regions become less cellular. Moreover, sequential “waves” of colonization by stem cells could also account for the non-uniform appearance of the thymic cortex. Secondly, it is clear that thymic epithelial differentiation (manifested as keratinization of epithelial cells in the medulla) was seen in reconstituted thymi from these dogs. Epithelial keratinization is not a regular feature of the gnotobiotic XSCIDs epithelial thymus. This finding suggests that T-cells also influence epithelial structure and function. The immunologic significance of this, if any, is not known. Finally, there are follicular (B-cell) structures present in the thymic medullas (Fig. 2, right panel). These are presumably of host (canine) origin as canine Ig-positive plasma cells were demonstrated in these sections by immunohistochemistry (data not shown).

In the tonsils (Fig. 3), clear histologic evidence for lymphoid follicle (B-cell) and parafollicular (T-cell) development was discerned indicating that, in the tonsil at least, population of a secondary lymphoid organ had occurred in the transplanted dogs. The spleens contained lymphoid follicles and small periarteriolar lymphoid sheaths. Spleen section replicates were stained for canine IgM- and IgG-positive plasma cells: both spleens contained IgG-positive plasma cells, again suggesting that post-thymic human T-cells provided the necessary signals for B-cell maturation and Ig class switching in the transplanted dogs. No ileal Peyer’s patches were seen and intestinal sections were devoid of lymphocytic infiltrates into the lamina propria. As in XSCIDs dogs, reconstituted with canine marrow cells, neither gross nor histologic evidence for the development of lymph nodes were found in either animal successfully engrafted with human cells. Finally, small scattered lymphocytic and
granulomatous inflammatory cell infiltrates were found in the myocardia of both transplanted dogs. There was no evidence of myocardial insufficiency and the significance of this finding is not known.

2. Discussion and conclusions

The reconstitution experiment with human stem cells outlined above demonstrates the value of gnotobiots for the investigation of diseases of both veterinary and human biomedical importance. Our early experiments with gnotobiotic XSCIDs dogs demonstrated that the “failure to thrive” phenomenon, characteristic of immunodeficiency disease is not a clinical feature of disease expression under gnotobiotic conditions (Felsburg et al., 1999). Thus, the runting effect is attributable to continuous environmental insults rather than to an intrinsic feature of gamma chain insufficiency. As a practical matter, gnotobiotic conditions renders the immunosuppressive state prior to expansion and peripheralization of the marrow stem cell grafts of minimal consequence to the recipient during thymic colonization and subsequent peripheralization after transplantation. Thus, dose-titrations for the minimally effective dose of stem cells can be performed. This advantage is especially important when performing xenotransplantation experiments with human stem cells as described here. While the minimal duration before conventionalization or controlled exposure to environmental antigens has not yet been defined, it is likely to be longer than 3 months for dogs given the human xenotransplants, an interval which cannot be reliably sustained in a conventional environment. The gnotobiotic conditions of these experiments has also revealed that limited T-cell development can be accomplished in a thymus-independent fashion, suggesting that there is at least a subpopulation of T-cells which are responsive to the environment and

Fig. 3. Left panel: histologic appearance of an unengrafted canine XSCIDs tonsil. Note the paucity of cells at the base of the tonsillar crypts. Hematoxylin and eosin stain. Right panel: histologic appearance of appearance of an XSCIDs tonsil from a dog engrafted with human CD34+ cells 20 weeks previously. Note the increased cellularity in the tonsillar crypts and the formation of nascent B-cell follicles in this tissue. Hematoxylin and eosin stain.
do not require maturation within the thymic micro-
environment (Felsburg et al., 2003). Clearly, this
pathway is insufficient to sustain life but its existence
certainly suggests avenues of study which may
enhance development of these cell type(s).

These experiments also highlight the potential
role(s) of immune (T) cells in epithelial cell
differentiation and, as well, the creation of lymph
nodes during embryogenesis. Clearly, normal lymph
node development must require interaction between
functional T-cells and B-cells during embryogenesis
prior to birth. Murine studies have convincingly
demonstrated that products of B-cells (LT, tumor
necrosis factor alpha and perhaps other chemokines)
are critical for the genesis of lymph nodes and for
proper development of B-cell follicles in these organs
(Chaplin and Fu, 1998; Ruddle, 1999). In dogs, the
thymus becomes cellular during the second “trime-
ster” of development at 35–45 days of gestation and
fetal pups are generally regarded as capable of making
both humoral and cellular immune responses at 50–55
days of gestation, after significant post-thymic
peripheralization of T-cells has occurred (Bryant
and Shifrine, 1972). In XSCIDs dogs, the thymus
does not colonized with pre-thymic T-cells and mature T-
cells are not produced. Thus, it must be that both the
prenatal population (CD4+, CD8+ or both) of
circulating T-cells and their interactions with develop-
ing B-cells is required for the initiation of peripheral
lymph node formation. Further, once this “window”
of development in embryogenesis is missed, normal
lymph node development likely will not occur even
when functional T-cells are provided to B-cells in the
reconstituted XSCIDs dogs.

That thymic development of human CD34-origin
pre-thymic T-cells occurred in the two XSCIDs dogs
which received the xenotransplants was indicated by
the:

(1) increased cellularity of the thymus;
(2) relative lymphocytosis versus untransplanted
control XSCIDs dogs and;
(3) flow cytometry analyses performed on PBMC
from these two dogs which documented the
appearance of human CD4+ circulating lympho-
cytes, albeit low numbers, which were also
marked with a monoclonal specific for human
CD45RA+ antigen.

This apparent success must be balanced against
some potential complications. Classically, the thymus
is not considered to be a location where immune
responses are initiated. Yet, the presence of lymphoid
follicles in the thymic medullae in both dogs suggests
that there are some potential host versus graft rejection
issues to consider. To our knowledge, thymic B-cell
follicles have been reported to occur in association
with two canine conditions. Gnotobiotic dogs, which
are convalescing from canine distemper virus infec-
tion, will display follicles in the thymi (McCullough
et al., 1974) and a local response to viral antigen(s)
most likely accounts for this effect. Dogs affected with
systemic lupus erythematosus (SLE) also develop
follicles in the thymi (Lewis and Schwartz, 1971). In
this instance, it is likely that this is a reflection of
generalized B-cell origin autoimmunity characteristic
of SLE. In the transplanted dogs, the “best guess
stimulus” for the appearance of thymic follicles in this
location is that there is some host B-cell responses,
possibly through the production and release of B
lymphocyte stimulator (BlyS) from dendritic cells and
monocytes in the thymus (Moore et al., 1999). It is
possible that the myocardial inflammatory infiltrates
are also reflections of the same phenomenon.

Finally, we do not know if the xenotransplanted
dogs are immunocompetant. Demonstration of IgG-
positive plasma cells in the spleens of these dogs
suggest that the human T-cells may provide “help” to
canine B-cells although it must be admitted that this
response may be partially independent of T-cell
facilitation (Moore et al., 1999). We did not perform
immunization experiments with them lest developing
immunity to experimental antigen(s) would complicate
interpretations of both the flow cytometric and
histologic data. Ultimately the best proof for the
efficacy of xenotransplantation lies in both the ability
of reconstituted human–dog chimeras to respond to
planned immunizations with modified live canine
distemper virus vaccine and also to the effects of the
environmental antigen load once transplanted dogs are
removed from the isolation units and held in a
conventional cage environment. We do not know if
human differentiated T-cells will respond properly to
dog-origin trafficking signals, cytokines or other
signaling pathways contained within the canine
milieu. Until the conventionalization and immuniza-
tion experiments are done, we do not know if we have
created a truly useful and innovative animal model of human lymphopoiesis or if instead we have created an immunologic enigma of minimal biological significance. With gnotobiotics, there is every reason to expect that our approach will be ultimately be successful provided that the transplanted dogs can be protected from environmental pathogens prior to maturation and proliferation of engrafted human cells.

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References